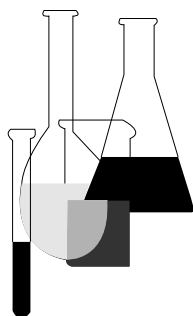




Ecological Effects Test Guidelines

OPPTS 850.2200 Avian Dietary Toxicity Test



“Public Draft”

INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

Public Draft Access Information: This draft guideline is part of a series of related harmonized guidelines that need to be considered as a unit. *For copies:* These guidelines are available electronically from the EPA Public Access Gopher (gopher.epa.gov) under the heading “Environmental Test Methods and Guidelines” or in paper by contacting the OPP Public Docket at (703) 305-5805 or by e-mail: guidelines@epamail.epa.gov.

To Submit Comments: Interested persons are invited to submit comments. By mail: Public Docket and Freedom of Information Section, Office of Pesticide Programs, Field Operations Division (7506C), Environmental Protection Agency, 401 M St. SW., Washington, DC 20460. In person: bring to: Rm. 1132, Crystal Mall #2, 1921 Jefferson Davis Highway, Arlington, VA. Comments may also be submitted electronically by sending electronic mail (e-mail) to: guidelines@epamail.epa.gov.

Final Guideline Release: This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on *The Federal Bulletin Board*. By modem dial 202-512-1387, telnet and ftp: fedbbs.access.gpo.gov (IP 162.140.64.19), or call 202-512-0135 for disks or paper copies. This guideline is also available electronically in ASCII and PDF (portable document format) from the EPA Public Access Gopher (gopher.epa.gov) under the heading “Environmental Test Methods and Guidelines.”

OPPTS 850.2200 Avian dietary toxicity test.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source material used in developing this harmonized OPPTS test guideline 40 CFR 797.2050 Avian Dietary Toxicity Test; OPP 71–2 Avian Dietary LC50 Test (Pesticide Assessment Guidelines, Subdivision E—Hazard Evaluation; Wildlife and Aquatic Organisms) EPA report 540/09-82-024, 1982; and OECD 205, Avian Dietary Toxicity Test.

(b) **Purpose.** This guideline designed to develop data on the dietary toxicity to bobwhite and mallard of chemical substances and mixtures subject to acute environmental effects test regulations. This guideline gives specific guidance for the testing of bobwhite and mallard, which are EPA's preferred test species. However, other species, such as pigeon, *Columba livia*, Japanese quail, *Coturnix coturnix japonica*, ring-necked pheasant, *Phasianus colchicus*, and red-legged partridge, *Alectoris rufa*, are also acceptable. The Agency will use these and other data to assess the acute hazard to birds and to provide an indication of potential chronic hazard that these chemicals may present to the environment.

(c) **Definitions.** The definitions in section 3 of the Toxic Substances Control Act (TSCA) and 40 CFR Part 792—Good Laboratory Practice Standards apply to this test guideline. In addition, the following definitions apply to this guideline:

Acclimation is the physiological or behavioral adaptation of test animals to environmental conditions and basal diet associated with the test procedure.

Basal diet is the food or diet as it is prepared or received from the supplier, without the addition of any carrier, diluent, or test substance.

Exposure period is the 5-day period during which test birds are offered a diet containing the test substance.

Hatch is eggs or young birds that are the same age and that are derived from the same adult breeding population, where the adults are of the same strain and stock.

LC50 is the empirically derived concentration of the test substance in the diet that is expected to result in mortality of 50 percent of a population of birds which is exposed exclusively to the treated diet under the conditions of the test.

Postexposure period is the portion of the test that begins with the test birds being returned from a treated diet to the basal diet. This period

is typically 3 days in duration, but may be extended if birds continue to die or demonstrate other toxic effects.

Test period is the combination of the exposure period and the postexposure period, or the entire duration of the test.

Test substance is the specific form of a chemical or mixture of chemicals that is used to develop the data.

(d) **Test procedures—(1) Summary of test.** (i) Birds should be acclimated for at least 7 days after they have been obtained.

(ii) Test birds should be randomly assigned to the various treatment levels and controls.

(iii) Definitive test concentrations should be established, possibly requiring a range-finding test to be conducted first.

(iv) The test substance should be mixed thoroughly and evenly into the diet. Three treatment levels should be analyzed for test substance concentrations.

(v) Birds should be weighed at the beginning and the end of the exposure period.

(vi) Birds should be observed regularly for mortality or abnormal behavior and any findings should be reported.

(vii) Food treated with the test substance should be replaced by untreated food (basal diet) after 5 days of exposure. Food consumption during the exposure period should be carefully estimated on a pen by pen basis.

(viii) Food consumption should be estimated for the postexposure period and birds should be weighed at the end of 8 days. Additional weights and food consumption estimates should be determined if the test period is longer than the typical 8 days.

(ix) The mortality pattern should be examined, and a statistical analysis should be conducted. The LC50, 95 percent confidence limits, and the dose-response slope should be reported. A test for heterogeneity of data should be conducted.

(x) Treated or positive control birds should be sacrificed and disposed of properly. Negative control birds may be kept as breeding stock, but should not be used in any other tests.

(xi) The material to be tested should be the highest purity available (technical grade or analytically pure) and the degree of purity should be reported along with the percentage of each impurity. If specifically required, a particular substance or mixture should be tested.

(xii) A test is unacceptable if more than 10 percent of the control birds die during the test.

(2) **Prerequisite data.** These include water solubility, vapor pressure, and the chemical stability of the test substance in water and light to ensure uniform mixtures and the stability of test concentrations.

(3) **Range-finding test.** Unless the approximate toxicity of the test substance is known already, a range-finding test should be conducted to determine the test substance concentrations to be used in the definitive test. (Refer to paragraph (d)(4)(iii) of this guideline for details on concentrations for definitive tests.) Procedures for range-finding tests may vary, but generally, groups of a few birds are fed three to five widely-spaced concentrations for 5 days. A concentration series of 5, 50, 500, and 5,000 ppm is suggested. The results of the range-finding test then may be used to establish the definitive test concentrations.

(4) **Definitive test—(i) Controls.** (A) A concurrent control is required during every test. The control birds should be from the same hatch as the test groups. Control and test birds should be kept under the same experimental conditions. The test procedures should be the same for control and treated birds, except that no test substance should be added to the diets of control birds. If a carrier is used in preparation of the test diets, the same carrier should be added to the diets of control birds in the highest concentration used for test diets. The use of shared controls is acceptable for concurrent tests as long as the same carrier is used for all the tests.

(B) Test acceptability criteria are as follows:

(1) A test is not acceptable if more than 10 percent of the control birds die during the test period.

(2) There must be evidence that the concentration of the substance being tested has been satisfactorily maintained in the diet (it should be at least 80 percent of the nominal concentration) throughout the first 5 days of the test period.

(3) The lowest treatment level should not result in compound-related mortality or other observable effects.

(C) A positive control (e.g. dieldrin standard) may be run, but is not required for each test. However, a quarterly or semiannual laboratory standard (positive control) is recommended as a means of detecting possible interlaboratory or temporal variation. A laboratory standard is also recommended when there is any significant change in food, housing, or source of birds.

(ii) **Number of animals tested.** In the definitive test, a minimum of 10 birds should be used for each dietary concentration of the test substance. A minimum of 20 birds should be used for the negative or carrier

control; 30 or more control birds are preferable. If a positive control or laboratory standard is used, 10 or more birds should also be used for each concentration of the positive control. When a test substance is known or expected to result in high experimental variation, it may be appropriate or required to use additional birds.

(iii) **Concentrations and dosage-mortality data.** A minimum of five concentrations of the test substance should be used in the definitive test. These concentrations should be spaced geometrically. The recommended spacing is for each concentration to be at least 60 percent of the next higher dose (less than 1.67 times the next lower dose). If concentrations are spaced more widely than is recommended, then at least three concentrations should result in mortality between, but not including, 0 percent and 100 percent. For any concentration spacing, at least one concentration should kill more than 50 percent (including 100 percent) and at least one concentration should kill less than 50 percent (including 0 percent) of the birds in a pen. For some test substances, it may be necessary to use more than five concentrations to achieve these results. The lowest test concentration should not result in test substance-related mortality or other observable effects.

(iv) **Duration of test.** The definitive test should include 5 days of exposure to the test substance in the diet (exposure period) followed by at least 3 days of additional observation (postexposure period) while the test birds are receiving an untreated diet. If any test birds die during the second or third day of the postexposure period or if toxic signs are evident on the third day of the postexposure period, the test period should be extended until 2 successive mortality-free days and 1 day free of toxic signs occur, or until 21 days after beginning the test, whichever comes first.

(v) **Observations of record.** (A) Throughout the test period, all signs of intoxication, other abnormal behavior, and mortality should be recorded and reported by dose level and by day. Signs of intoxication are those behaviors apparently due to the test chemical and may include a wide array of behaviors, such as labored respiration, leg weakness, hemorrhage, convulsions, ruffled feathers, etc. All signs of intoxication and any other abnormal behavior, such as excessive aggression, toe-picking, etc., that may or may not be attributed to the test substance should be reported. Among survivors, remission of signs of intoxication and cessation of abnormal behavior should be recorded by dose level and by day. When differential signs of intoxication are observed within a dose level, an estimate of the number of birds exhibiting such signs should be recorded. Observation of test birds should be made, at a minimum, 3 times on the first day of the exposure period. Observations also should be made at least daily throughout the remainder of the test period; twice daily observations are recommended, where feasible.

(B) Average body weights of birds should be recorded and reported for each pen within each treatment and control group at the beginning of the exposure period and the end of the normal 3-day postexposure period of each test. Body weights 72 h before the exposure period are not required, but would provide valuable base-line data. Average food consumption should be measured in control pens and pens with the second lowest and second highest concentration levels either daily or every other day. Any significant amount of food spilled onto litter pans should be estimated and reported. For all other pens, average food consumption should be measured for both the exposure period and the normal 3-day postexposure period. If the study is continued beyond 8 days, body weight and food consumption data should be recorded weekly.

(C) Gross pathology examinations are not required, but they may provide valuable information on target site, mode of action, etc.

(5) **Analytical measurements**—(i) **Statistical analysis.** (A) A statistical analysis should be conducted by transforming the dietary concentrations to logarithmic values and the mortality pattern to probits. Other acceptable methods that will result in a theoretically straight line through ± 2 standard deviations from the LC50 value may be used. The LC50 value and slope of the transformed concentration-response curve should be determined for mortality at the end of test period. Probit analysis by calculations or graphical probit methods are preferred. Any standard method that is used should provide the slope of the transformed concentration-response curve as well as the LC50 value. A statistical test for goodness-of-fit (e.g. X^2 test) also should be performed. When mortality at the level of 5,000 ppm, the highest recommended treatment level, is less than 50 percent and the LC50 cannot be calculated, the LC50 should be reported as greater than 5,000 ppm, and the no-effect level reported as well.

(B) All methods used for statistical analysis should be described completely.

(ii) **Analysis for test substance concentrations.** (A) Samples of treated diets should be analyzed to confirm proper dietary concentration of the test substance. Analyses should be conducted at the beginning of the exposure period with samples from high, middle, and low concentrations. If not already available, data should be generated to indicate whether or not the test substance degrades or volatilizes. If the test substance is known or found to be volatile or labile to the extent that 25 percent or more loss occurs over a 5-day period, then a second series of analyses of the same concentrations previously analyzed should be conducted at the end of the exposure period.

(B) The assay method used to determine actual concentrations should be reported.

(C) If it is observed that the stability or homogeneity of the test substance in the diet cannot be maintained, care should be taken in the interpretation of the results and note made that the results may not be reproducible.

(iii) **Analysis of basal diet.** A nutrient analysis of the basal diet should be included in the test report. For commercially prepared basal diets, the list of ingredients supplied by the company is normally sufficient if it is detailed. The composition of any vitamin or other supplements should also be reported.

(e) **Test conditions**—(1) **Test species**—(i) **Selection.** (A) An upland bird, bobwhite quail, *Colinus virginianus* (L.), and a waterfowl, mallard duck, *Anas platyrhynchos* L., are the preferred test species. Birds may be reared in the laboratory or purchased from a breeder. If bobwhite are purchased, it is preferable that they be obtained as eggs which then are hatched and reared in the testing facility. During incubation, a temperature of 39 °C and relative humidity of 70 percent are recommended for bobwhite. It is feasible to purchase live young bobwhite chicks if they can be obtained locally, however, young bobwhite may suffer adverse effects if shipped by air or other commercial means. Young mallard ducklings normally can be shipped without undue adverse effects.

(B) All control and treatment birds used in a test should be from the same source and hatch. Birds should be obtained only from sources whose colonies have known breeding histories. Birds should be phenotypically indistinguishable (except for size) from wild stock. It is recommended that birds be obtained from flocks that have been outbred periodically with genetically wild stock in order to maintain a genetic composition that approximates the natural heterogeneity of the species.

(C) Birds used in the test should be in apparent good health. Deformed, abnormal, sick, or injured birds should not be used. During the 72-h period preceding testing, the health of the populations should be monitored and mortalities recorded. Birds should not be used for a test if more than 5 percent of the total test population die during the 72 h immediately preceding the exposure period. Purchased birds should be certified as disease-free or as bred from disease-free stocks. Birds should not have been selected in any way for genetic resistance to toxic substances. Birds should not have been used in a previous test, either in a treatment or control group.

(D) Young birds should be tested to ensure that test birds must feed during the exposure phase of the test. Mallards should be 5 to 10 days old and bobwhite should be 10 to 14 days old at the beginning of the exposure period. All treatment and control birds in a test should be the same age ± 1 day. The exact age should be recorded and reported.

(E) Test birds should be acclimated to test facilities and basal diet for a minimum of 7 days. Acclimation to test pens may be either in the actual pens used in the test or in identical pens. Birds used in the test should be assigned randomly to treatment and control pens without respect to sex. Randomization may be done either at the initiation of the acclimation period or at the time when the birds are weighed at the beginning of the exposure period.

(F) Birds should be shielded from excessive noise, activity, or other disturbance during holding, acclimation, and testing. Birds should be handled only as much as is necessary to conform to test procedures.

(ii) **Diet.** (A) A standard commercial game bird (for bobwhite) or duck (for mallard) starter mash, or the nutritional equivalent, should be used for diet preparation. Antibiotics or other medication should not be used in the diet before or during the test. For bobwhite only, an antibiotic demonstrated to fully depurate in 72 h may be added to the drinking water, if necessary, for birds up through 10 days of age. Only clean unmedicated water should be offered during the 96 h preceding the exposure period and during the test period. It may not be possible to obtain food that is completely free of pesticides, heavy metals, and other contaminants. Diets should be analyzed periodically, and should be selected to be as free from contaminants as possible. A nutrient analysis and list of the ingredients in the diet should be included with the test report.

(B) The test substance should be mixed into the diet in a manner that will result in even distribution of the test substance throughout the diet. If possible, the test substance should be added to the diet without the use of a diluent. If a diluent is needed, the preferred diluent is distilled water, but water should not be used as a diluent for test substances known to hydrolyze readily. When a test substance is not water soluble, it may be dissolved in a reagent grade evaporative diluent (e.g. acetone or methylene chloride) and then mixed with the test diet. The diluent should be completely evaporated prior to feeding. Other acceptable diluents may be used, if necessary, and include table grade corn oil, propylene glycol, and gum arabic (acacia). If a diluent is used, it should not comprise more than 2 percent by weight of the treated diet, and an equivalent amount of diluent should be added to control diets for untreated birds.

(C) Diets can be mixed by commercial, mechanical food mixers. For many test substances, it is recommended that treated diets be mixed under a hood. Mash and test substances should be mixed freshly just prior to the beginning of the test. Certain volatile or other test substances may require preparation of fresh diets at frequent intervals. Analysis of the diet for test substance concentrations is required under paragraph (d)(5)(ii) of this guideline.

(D) Clean water should be available *ad libitum*. Water bottles or automatic watering devices are recommended. If water pans or bowls are used, water should be changed at least once a day.

(2) **Facilities.** (i) Tests should be conducted with birds being maintained in commercial brooder pens or pens of similar construction. Pens should be constructed of galvanized metal, stainless steel, or perfluorocarbon plastics. Materials that are toxic, may affect toxicity, or may adsorb test substances should not be used. Wire mesh should be used for floors and external walls; solid sheeting should be used for common walls and ceilings. Wire mesh for floors should be fine enough so as to not interfere with the normal movement of young birds. Pens for housing young birds should have a floor area of at least 300 cm²/bird (approximately 50 in²/bird) for bobwhite quail and at least 600 cm²/bird (approximately 100 in²/bird) for mallards. Pens should be disassembled (if feasible) and should be cleaned thoroughly between tests. Steam cleaning of cages is recommended. Cages may be brushed thoroughly, as an alternative method. The use of detergents or bleach is acceptable, but other chemical disinfectants such as quaternary ammonium compounds should not be used. When necessary to control disease vectors, hot or cold sterilization techniques are recommended, as long as such techniques will not leave chemical residues on the cages. For cold sterilization, ethylene oxide is recommended. Pens should not be cleaned during a test.

(ii) Pens should be kept indoors to control lighting, temperature, and other environmental variables. Pens should be heated, preferably by thermostatic control. A temperature gradient in the pen of approximately 38 °C to approximately 22 °C will allow young birds to seek a proper temperature. Temperature requirements for young birds typically decline over this range from birth through the first several weeks of life. Relative humidity is not as critical, but the test room should be maintained at a relative humidity of 45–70 percent. A photoperiod of 14 h light and 10 h dark is recommended. Other light/dark cycles should not be used, but continuous lighting is acceptable. Lighting may be either incandescent or fluorescent. Pens and lights should be positioned so that all pens will receive similar illumination. The facilities should be well ventilated.

(iii) Where feasible, it is recommended that pens not be stacked upon each other. If pens are stacked, only one test substance is allowed in any single stack. If a test substance volatilizes or otherwise forms aerosols or vapors in the air, no more than one test substance should be tested in a room in order to avoid cross-contamination. Pens should be randomly arranged, whether or not in a stack, with respect to dose levels and controls. Pens, such as stacked, unmodified, commercial pens with external feeders, that allow food to be spilled from one pen to a lower pen, should be avoided. Any modifications that prevent cross contamination of concentration levels are acceptable. For example, commercially available, 30 cm (1 ft) long chick feeders may be placed inside the pens and be

covered with 1.27 cm (0.5 in) mesh hardware cloth over the food, for bobwhite. The same feeders covered with approximately 2.5 cm (1 in) mesh wire are appropriate for mallards. For either species, external feeders can be covered with the appropriate size wire mesh and a solid piece of metal extended from the bottom of the cage to a point exterior to the feeder. Spillage may occur, but the added metal will prevent food from spilling into another feeder.

(f) **Reporting.** (1) The test report should include the following information:

(i) Name of test, sponsor, test laboratory and location, principal investigator(s), and actual dates of beginning and end of test.

(ii) Name of species tested (including scientific name), age of birds (in days) at the beginning of the test, average body weights for birds in each pen at the beginning of the test, the end of the exposure period and end of the test, and individual weights of all birds that die during the test.

(iii) Description of housing conditions, including type, size, and material of pen, pen temperatures, approximate test room humidity, photoperiod, and lighting intensity.

(iv) Detailed description of the basal diet, including source, diluents (if used), and supplements (if used). A nutrient analysis of the diet should be included in the test report.

(v) Detailed description of the test substance including its chemical name(s), source, lot number, composition (identity of major ingredients and impurities), and known physical and chemical properties that are pertinent to the test (e.g. physical state, solubility, etc.).

(vi) The number of concentrations used, nominal and (where required) measured dietary concentration of test substance in each level, assay method used to determine actual concentrations, number of birds per concentration and for controls, and names of toxicants used for positive controls (if applicable).

(vii) Acclimation procedures and methods of assigning birds to test pens.

(viii) Frequency, duration, and methods of observation.

(ix) Description of signs of intoxication and other abnormal behavior, including time of onset, duration, severity (including death), and numbers affected in the different dietary concentrations and controls each day of the test period.

(x) Estimated food consumption per pen daily or every other day in the second highest and second lowest concentration and control pens. For other pens, food consumption should be estimated for the exposure period and for the postexposure period.

(xi) Location of raw data storage.

(xii) Results of range finding tests (if conducted).

(xiii) The calculated LC50 value, 95 percent confidence limits, slope of the concentration-response curve, the results of the goodness-of-fit test (e.g. X^2 test), and a description of statistical methods used. The same statistics are to be provided for positive controls (when used). The methods used for statistical analysis should be described completely.

(xiv) Anything unusual about the test, any deviation from these procedures, and any other relevant information.

(2) In addition to the above information required in every report, the following information should be available upon request:

(i) A general description of the support facilities.

(ii) A description of the quality control/quality assurance program, including the average quality level for the program element performing the test, procedures used, and documentations that these levels have been achieved.

(iii) The names, qualifications, and experience of personnel working in the program element performing the test, including the study director, principal investigator, quality assurance officer, as well as other personnel involved in the study.

(iv) Standard operating procedures for all phases of the test and equipment involved in the test.

(v) Sources of all supplies and equipment involved in the test.

(vi) Originals or exact copies of all raw data generated in performing the test.